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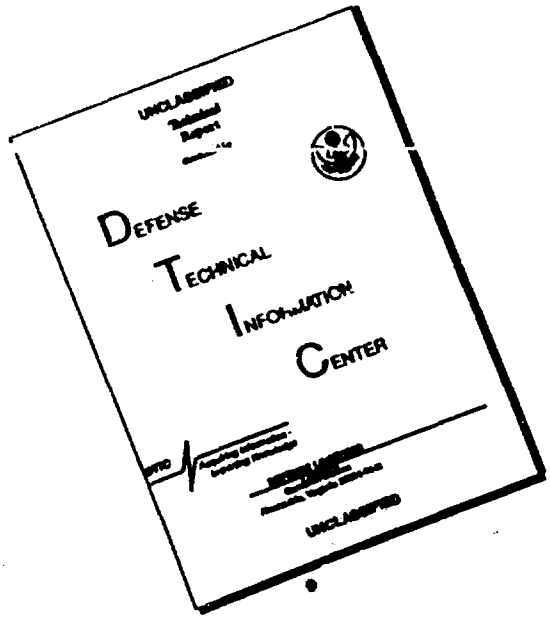

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CONTAMINATION OF TISSUE CULTURES WITH PLEUROPNEUMONIA-LIKE ORGANISMS

[Following is the translation of an article by I. V. Rakovskaya, Moscow Scientific-Research Institute for Viral Preparations, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology), No 2, 1965, pages 233-235. It was submitted on 6 May 63. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

In contemporary microbiological literature there are many reports on the contamination of transplantable cell lines of various origin with pleuropneumonia-like organisms (PPLO) - Mycoplasmataceae. Hearn et al. /6/ note that it is difficult to diagnose the contamination of cell lines with pleuropneumonia-like organsims for the following reasons: 1) mycoplasms usually do not cause any cytopathogenic changes in tissue cultures; 2) they cause only a very light or completely unnoticeable turbidity of liquid medium; 3) on agar media the growth of mycoplasms may remain unnoticed due to the minute colony size; 4) the majority of microorganisms in this group are resistant to penicillin and streptomycin - antibiotics usually used during the cultivation of tissues.

In the present report we will present data on the contamination of transplantable cell lines and the primary cultures with mycoplasms, we will describe several properties of these organisms and present the results from experiments on the purification of cell lines of mycoplasms.

For the isolation of pure cultures of mycoplasm we used 0.3% agar, prepared on tryptic digest of bovine heart with 10% noninactivated normal horse serum or serum from horned cattle.

The tissue cultures being investigated were cultivated by the generally accepted method. For determining the contamination of the tissue cultures 0.5--1 ml of cultural fluid, taken from cells grown in a continuous layer, were centrifuged for 10 minutes at 1000 rpm and then, using a Pasteur pipette, were inoculated in 10 ml of melted agar. The cultures were incubated under aerobic conditions at 37°. Colony growth was considered 4--6 days after inoculation.

Transplantable lines of cells and initially trypsinized cultures were investigated for contamination with mycoplasms.

All told 49 transplantable cell lines were investigated. These were obtained from various institutes. Forty-five of them (01%) turned out to be contaminated with mycoplasms. Thus only 4 lines were free from infection. The results of these investigations are presented in the table.

The mycoplasm strains, isolated from the various cell lines, grew in an agar layer in the form of minute colonies, which were slightly pigmented, uniformly compact, or had a dense center and delicate periphery. We did not detect any differences in the morphological structure of mycoplasm colonies, isolated from various cell lines. In connection with this, on the basis of morphological investigations alone it is not possible to identify the strains of mycoplasm contaminating the tissue cultures.

All the isolated mycoplasms grew in subcultures on agar medium and did not multiply in cell-less media No 199, Eagle's and 0.5% solution of lactalbumin hydrolyzate in Hank's salt solution in the presence of 10--20% horse serum or the serum of horned cattle, or 10--20% bovine amniotic fluid. The mycoplasms were preserved for 6 months in cell-less medium No 199 with 10% serum of horned cattle at 4°.

We investigated the following initially trypsinized cultures for mycoplasm contamination: Monkey kidneys, embryos of a pig, cow, man, embryo lungs of man and swine, chick fibroblasts. We were not able to isolate mycoplasms from the listed cultures.

Freeing tissue cultures of mycoplasms is extremely necessary for all virological investigations. In connection with this the efforts of many investigators have been directed to searching for the most active methods and means for decontaminating tissue cultures. Hayflick /5/ recommends that contaminated cultures be heated for 18 hours at 41° or 15--30 minutes at 44--45°. We heated the incubated contaminated cultures of HeLa, HEp-2 and SOTs in a medium which did not contain serum for various times and at various temperatures.

It turned out that the influence of high temperature did not free the contaminated cultures of mycoplasms. The mycoplasms survived even during prolonged heating at 45°. It was not possible to rid the contaminated cultures of mycoplasms with the help of freezing.

After 2 passages on a medium containing 500 AU of monomycin in 1 ml, cells of HEp-2, HeLa and SOTs went through 10 passages on medium without monomycin and remained free of mycoplasms. Thus this result testifies to the sufficiently persistent effect of monomycin as a substance for ridding tissue cultures of mycoplasms.

There are a number of reports by foreign authors concerning the successful use of Kanamycin in a concentration of 50 mg per 1 ml of medium for purifying cell lines of mycoplasms /9/. Levashev and Tsilinskiy obtained good and sufficiently persistent results when investigating the antibiotic Mycerin.

Conclusions

1. Out of the 49 transplantable cell lines investigated 45 (91%) ~~turned out to be~~ contaminated with mycoplasms.

2. The morphology of mycoplasm colonies, isolated from various tissue cultures, proved to be the same.

3. The influence of high and low temperatures did not rid contaminated cultures of mycoplasm.

4. With the help of monomycin it was possible to purify HEp-2, HeLa and SOTs cell cultures of mycoplasms.

Literature

1. Hayflick, ?., Ibid., 1960, v. 185, p 783.

2. Hearn, H. J. Jr., Officer, J. E., Elsner, V. et al., J. Bact., 1959, v. 78, p 575.

3. Rouse, H. C., Bobifas, V. H., Schlesinger, R. W., Virology, 1963, v.20, p 357.

Detection of mycoplasms in transplantable cell lines.

Culture	Detected in culture	Culture	Detected in culture
HEp-2	+	FK (fibroblast-like cells, obtained during trypsinization of capsule, limiting abscess in the lung of a grown rat	+
HeLa (line conducted on medium No 199 + 10% serum of horned cattle)	+	FL (cells obtained from the hospital im. F. E. Derzhinskogo)	+
HeLa (line conducted on medium No 199 and 10% human serum)	-	Fl (cells obtained from GORSES)	+
SOTs	+	M(a1)	+
KB	+	M(a2)	+
Detroit 6	+	M(a3)	+
A 16--45 (amniotic human cells obtained from Czechoslovakia)	+	M(a4) } cloned lines of KEM	+
SP (kidney cells from human embryo from Sverdlovsk Sci.Res. Institute)	+	M(a5)	
Chang's cells (from Moscow Institute of Viral Preparations)	+	J-96	+
Chang's cells (from Institute Virology imeni D. I. Ivanovskogo)		$J-97_{11}$	+
PaO (cells isolated from inguinal gland of Rhesus monkey)	+	$J-96_{12}$	-
SPE (kidney cells from pig embryo)	+	$J-96_{14}$	+
SPE-4	+	$J-96_{16}$	+
SPE-44 } cloned lines of SPE	-	$J-96_{32}$ } cloned lines of J-96	+
SPE-45	-	MiO (cells isolated from tonsil of Rhesus monkey)	+
SPE-46	+	A-1	
SPE (kidney cells of pig embryo, from the Central Control Institute)		A-1 - 59	
KEM	+	A-1 - 60	
		A-1 - 52	
		A-1 - 54	+
		A-1 - 24	
		A-1 - 38	
		A-1 - 23	
		A-1 - 19	
		A-1 - 37	
		A-1 - 63	
		A-1 - 61	
		A-1 - 42	
		A-1 - 62 } Amniotic human cells Cloned lines	